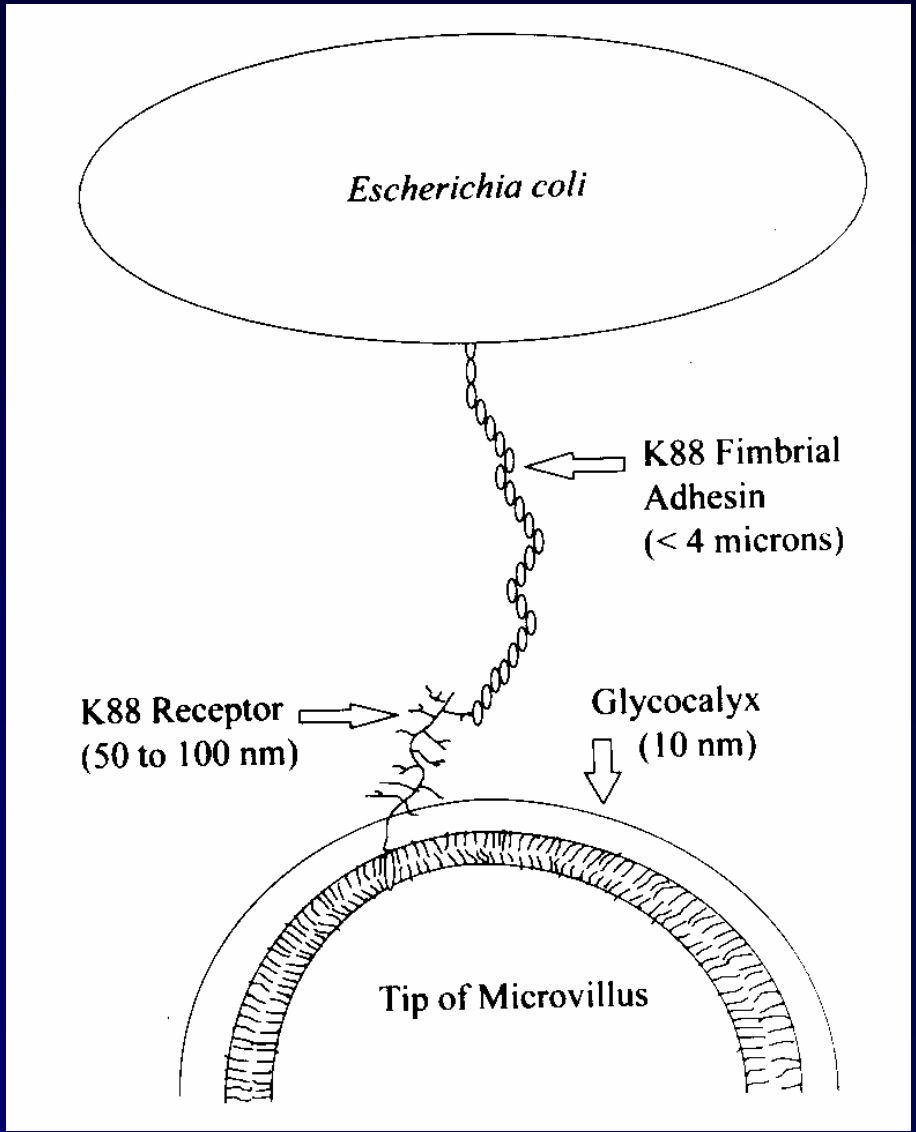
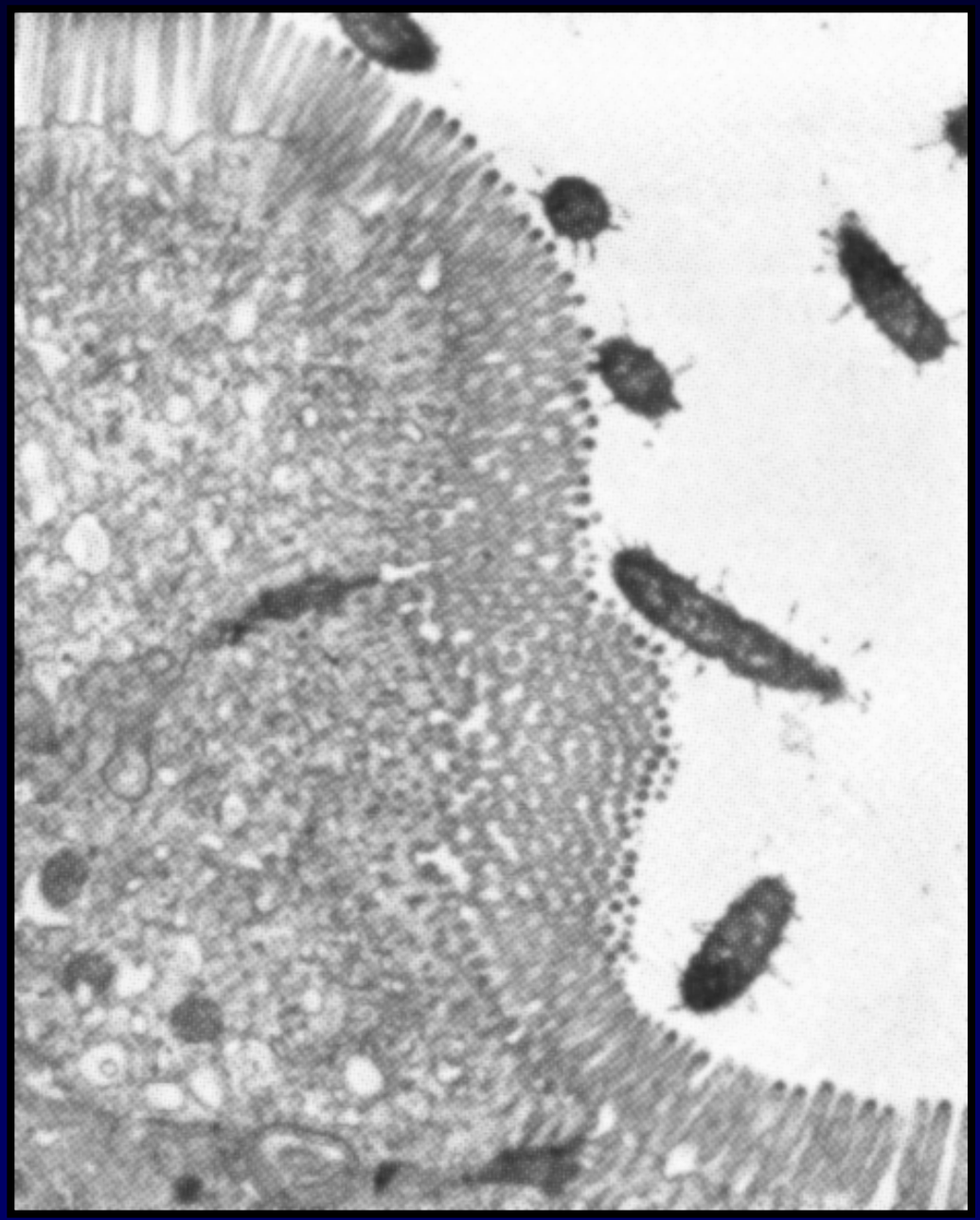
Exposure Sensitivity to Biofunctionalized Polymer-Based Nanoparticles

Robert A. Latour
Professor of Bioengineering
Clemson University

Bacterial Binding to Host is Mediated by Adhesins

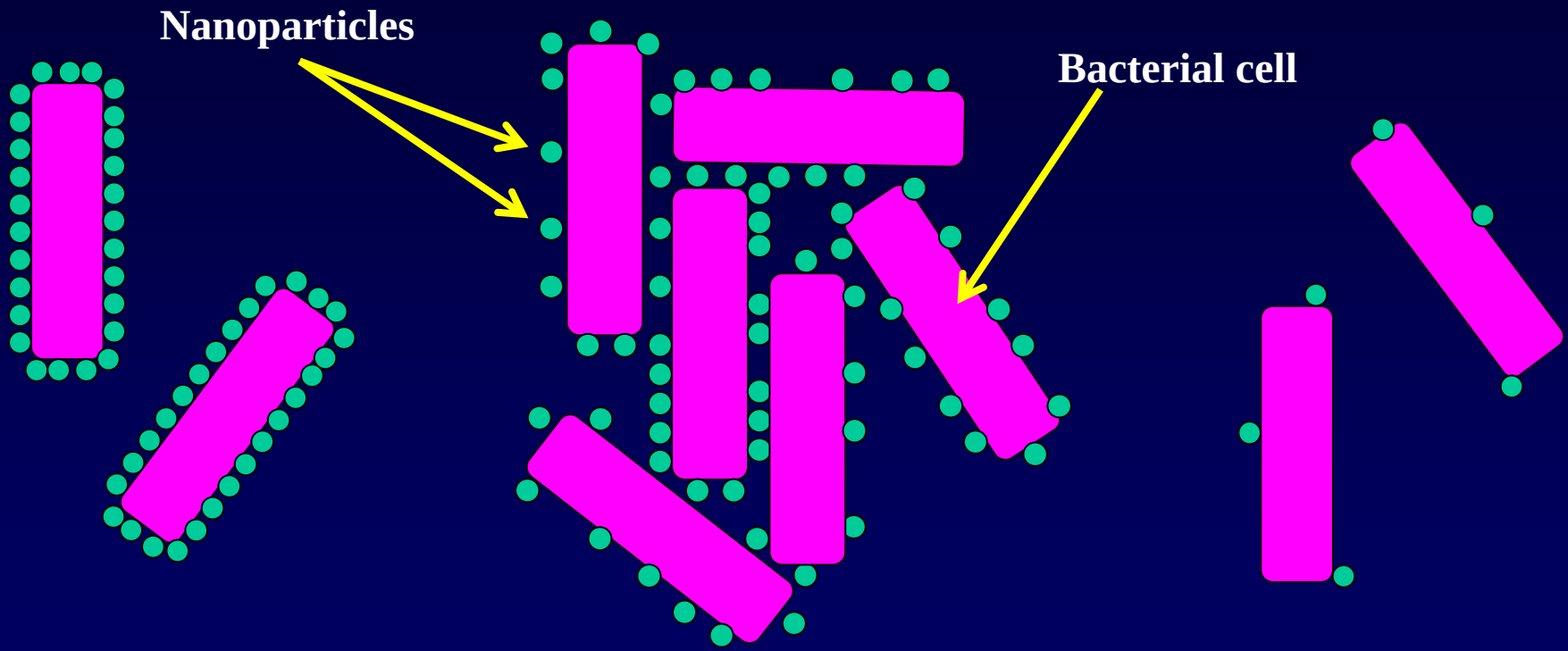




Transmission electron micrograph of *E. coli* adhering to epithelium in the intestine of a pig.

Moon, H.W. 1997. Comparative histopathology of intestinal infections. In: Mechanisms in the pathogenesis of enteric diseases (P.S. Paul, D.H. Francis and D.A. Benfield, eds.) Adv. Exptl. Med. Biol. 412:1. Plenum Press, New York.

Bacterial Cell Binding Strategies

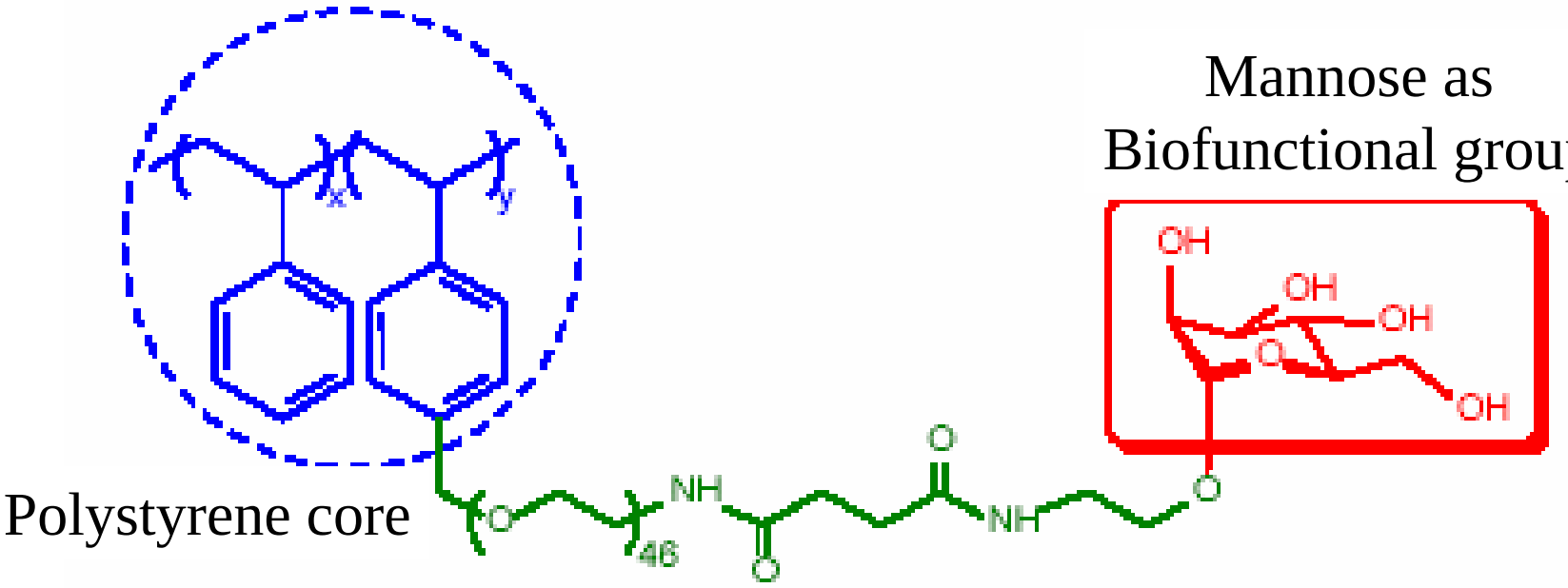


High NP Concentration: Bacterial Isolation

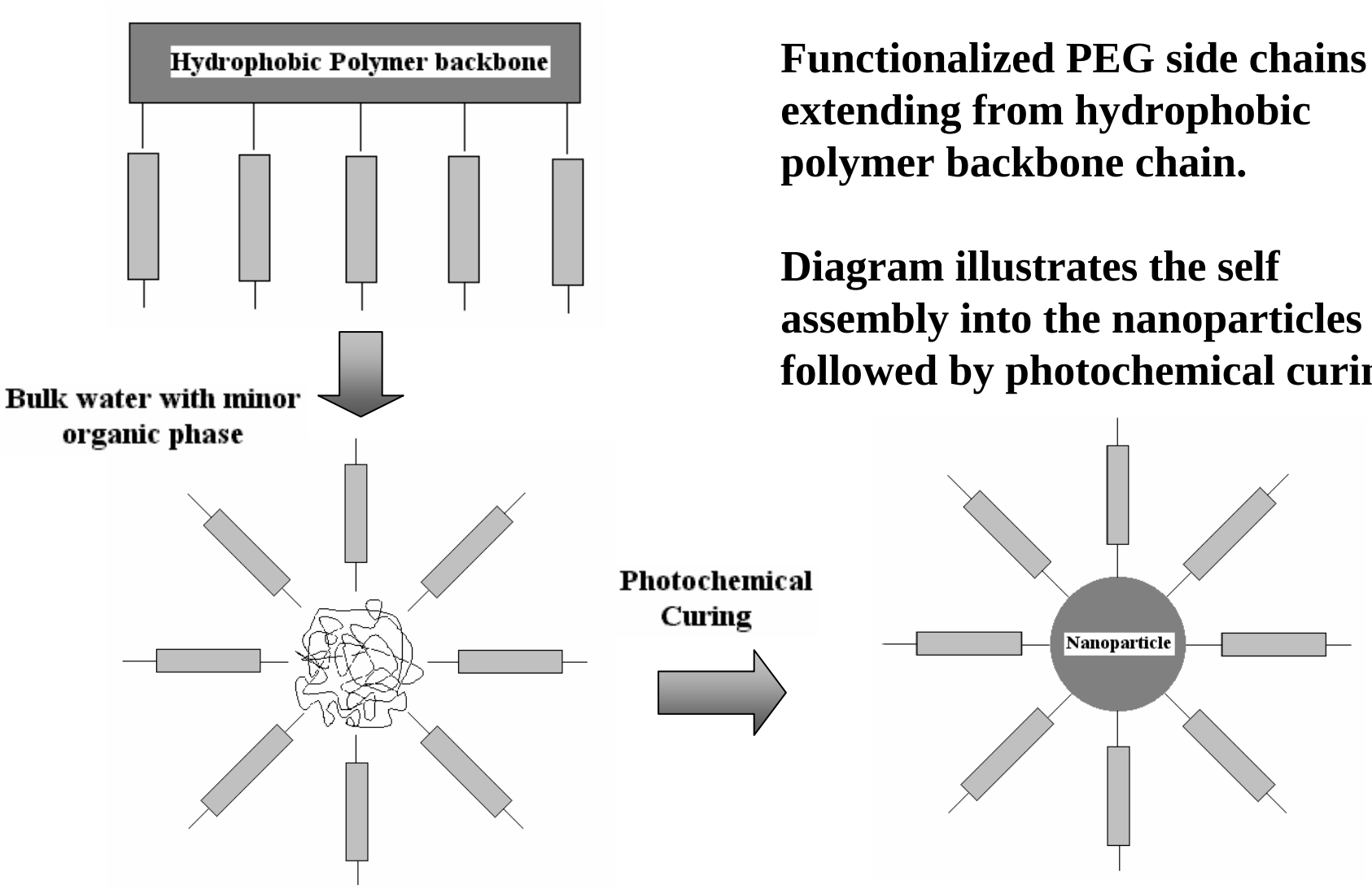
Intermediate NP Concentration: Bacterial Agglutination

Low NP Concentration: Bacterial Tagging

Nanoparticle Chemical Structure: Mannose Functionalization



Nanoparticle Design Strategy



Functionalized PEG side chains extending from hydrophobic polymer backbone chain.

Diagram illustrates the self assembly into the nanoparticles followed by photochemical curing.

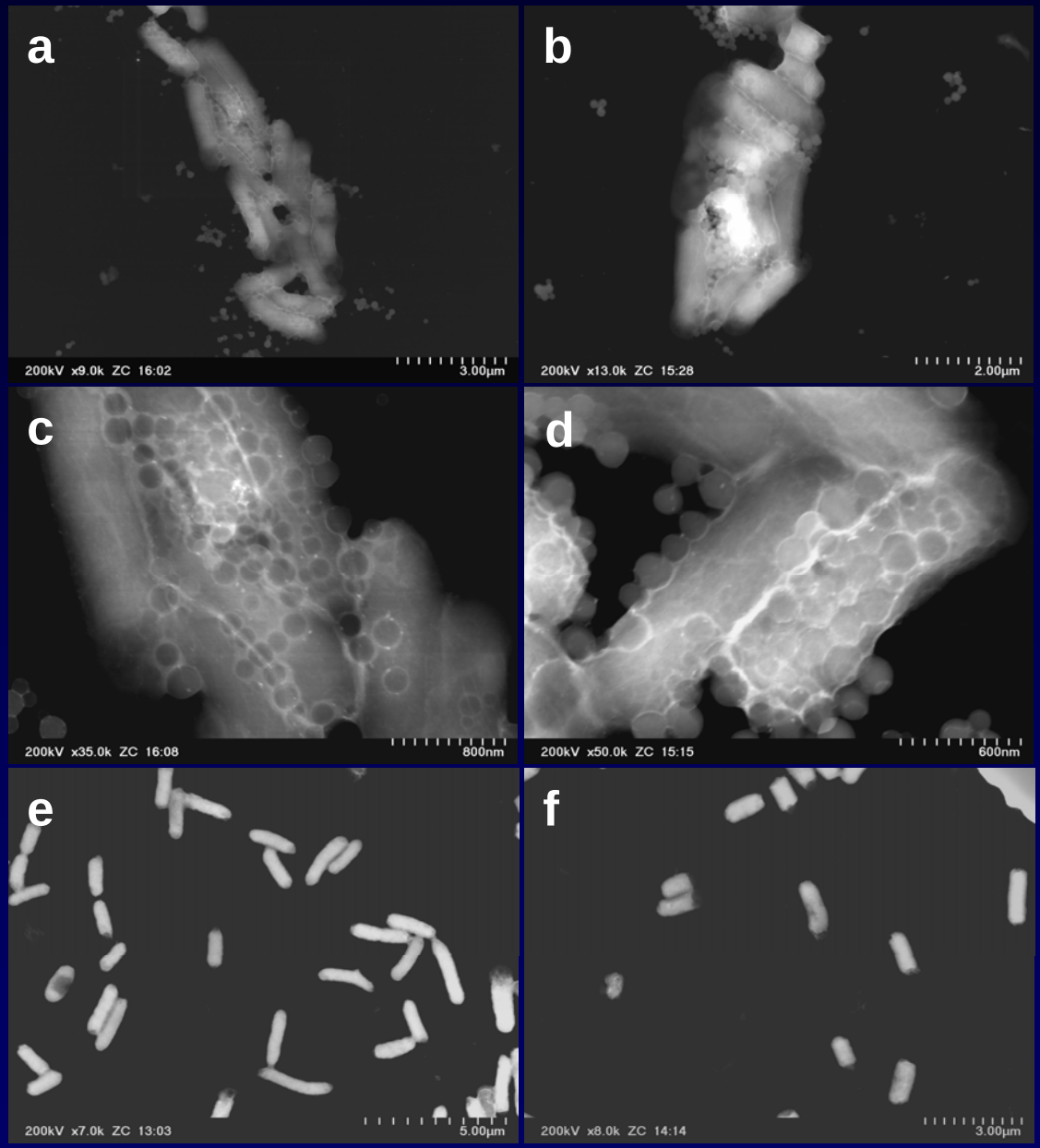
E. coli - NP Interaction

TEM images (dark-field) showing the agglutination of *E. coli* ORN178 mediated by D-mannose-tethered nanoparticles

(a,b) Lower magnification and (c,d) higher magnification

(e) *E. coli* ORN178 only (similarly with bare nanoparticles)

(f) *E. coli* ORN208 with the same D-mannose-tethered polymeric nanoparticles.



Acute Nanoparticle Exposure Sensitivity Studies

- In vitro studies
  - cell toxicity studies
- In vivo studies
  - Skin (rabbit)
  - Ocular (rabbit)
  - Inhalation (rat)
  - Ingestion (rat)
- In vivo studies: poultry

In Vitro Results: Dermal Fibroblasts

1 ml cells + medium / 50 µl 2wt% np solution (core-PEG np)

P = proliferating cells; NonP = nonproliferating cells

np = with nanoparticles; C = control (w/o np)

	Total Cell Count			
Trial	**P(C)**	**P(np)**	**NonP(C)**	**NonP(np)**
Mean (N=4):	**95,625**	**95,000**	**316,875**	**281,875**
95%CI:	29,476	28,865	86,619	35,779
p value:	**0.963 (not significant)**		**0.300 (not significant)**	

Dermal Test: Mannan Nanoparticles

Site preparation

Application of dose (1 mL, 2.0 wt.%)

Applying gauze

Overview after procedure

Results: Dermal Test (48 hrs)

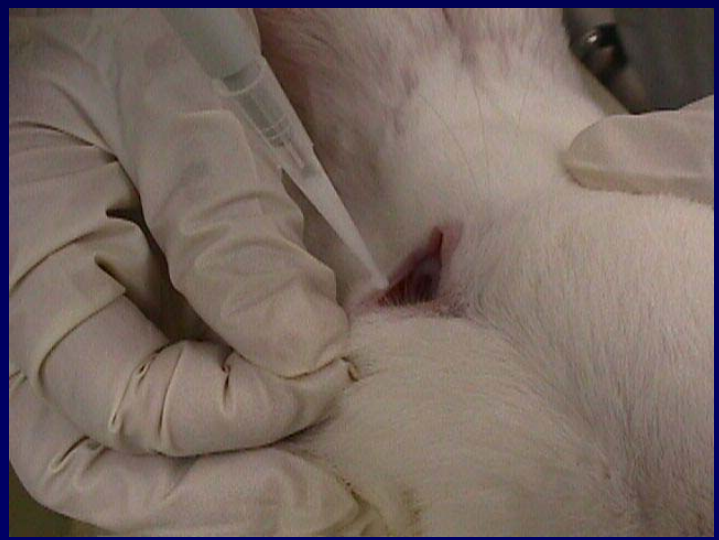
Control – no treatment

Test-plain solution

Test-nanoparticle solution

Ocular Test: Mannan Nanoparticles

Right and left eye before procedure



Application of dose (0.1mL at 2.0 wt.%)

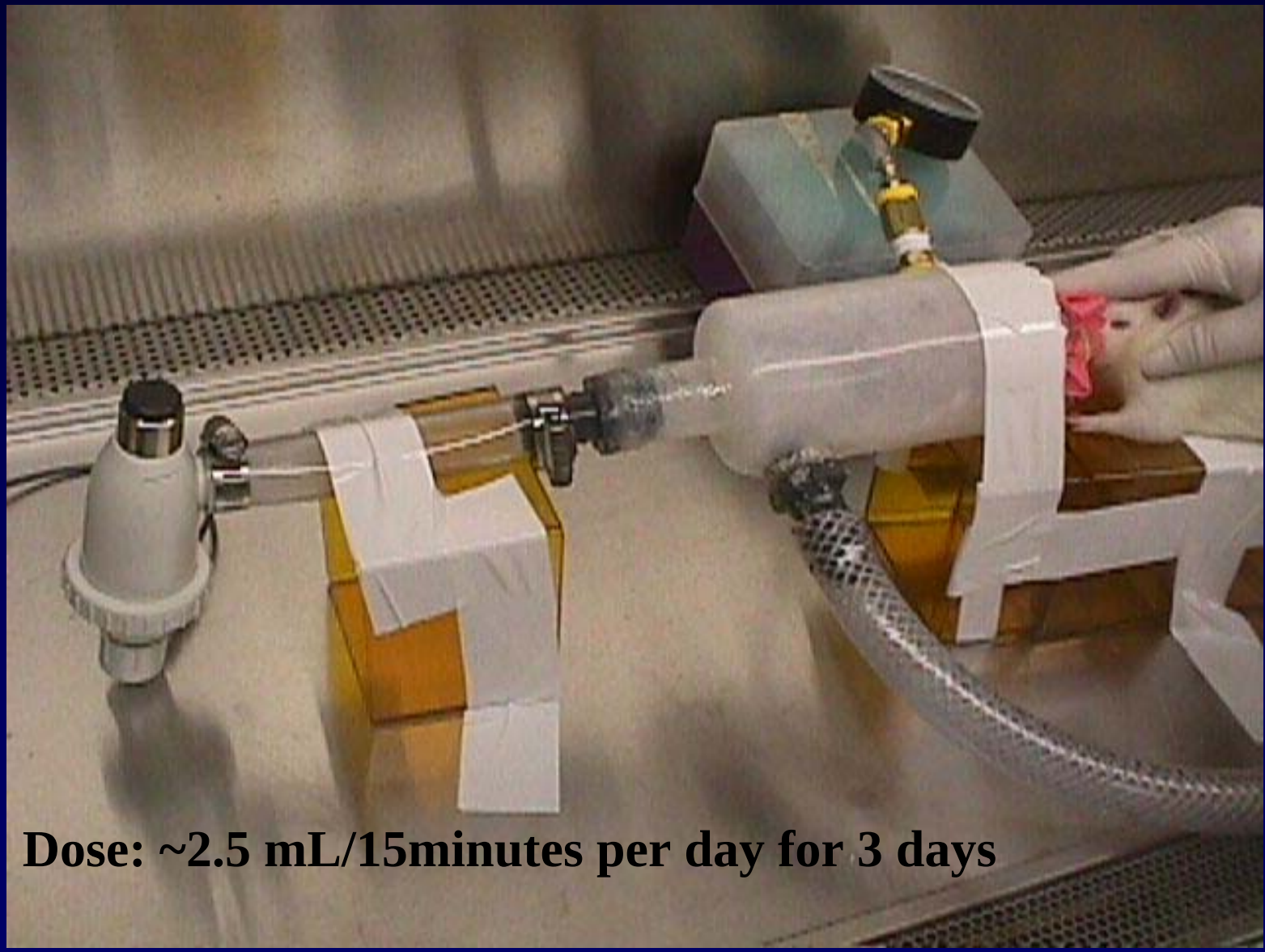
Right eye 1 min. after dose

Results: Ocular Test

48 hr.

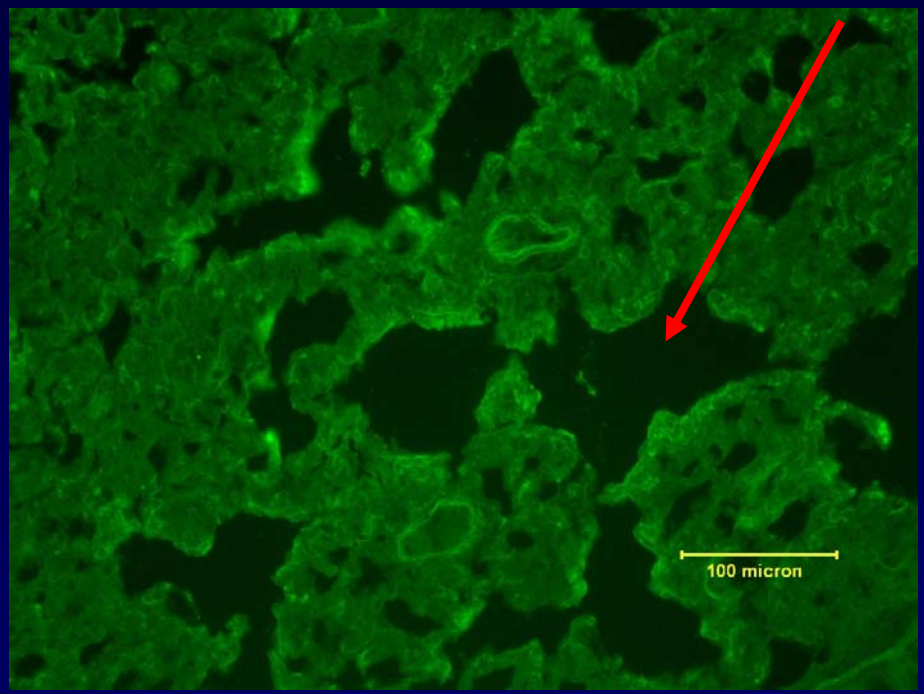
72 hr.

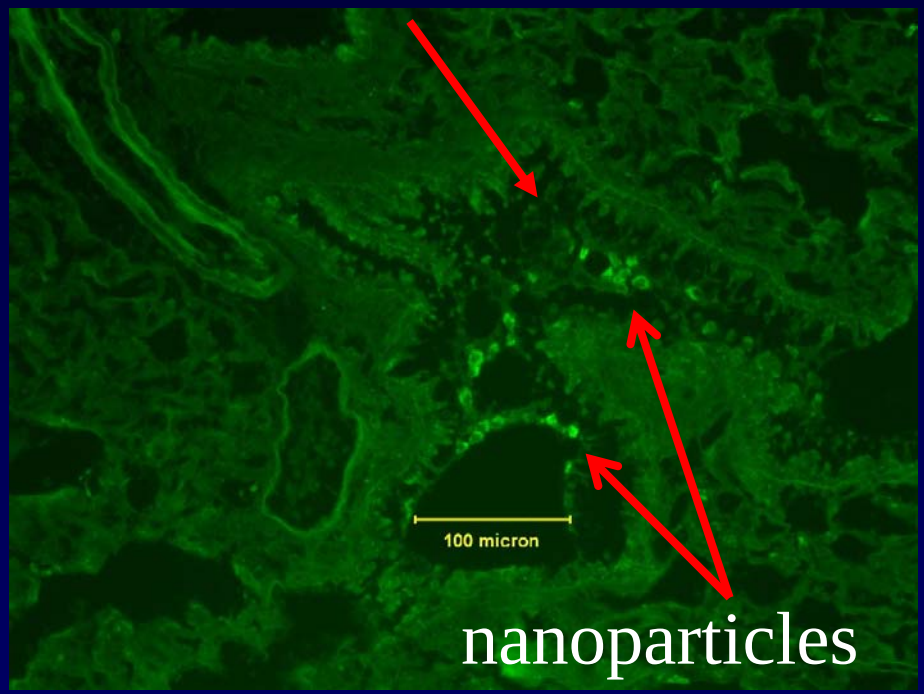
Inhalation Studies: FITC-labeled Mannan-NP



Inhalation Study: Lung Tissue (fluorescence) 72 hr.

Alveolar Sac / Alveolar duct



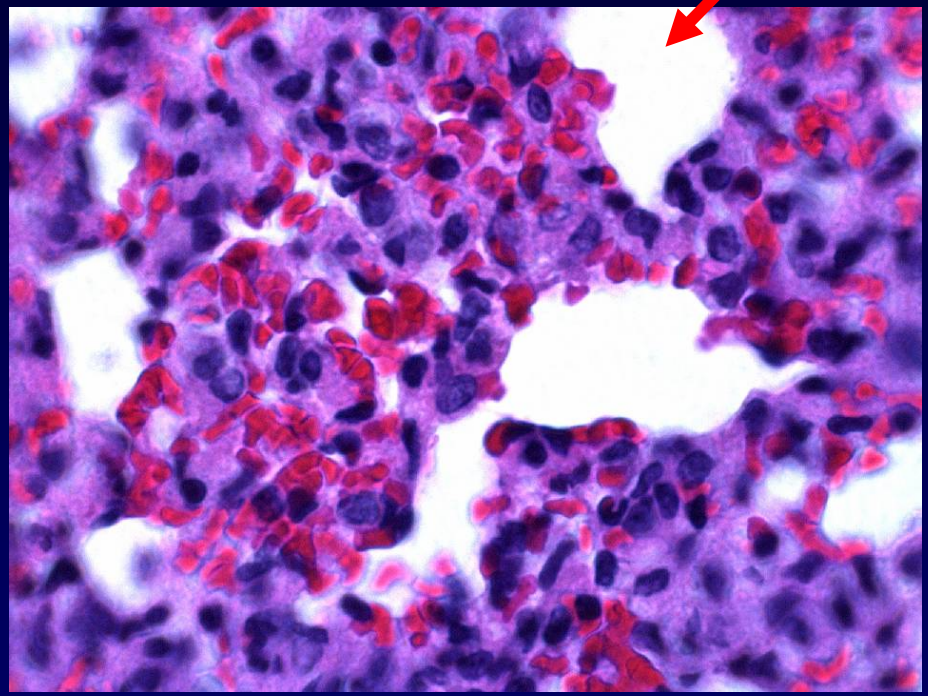


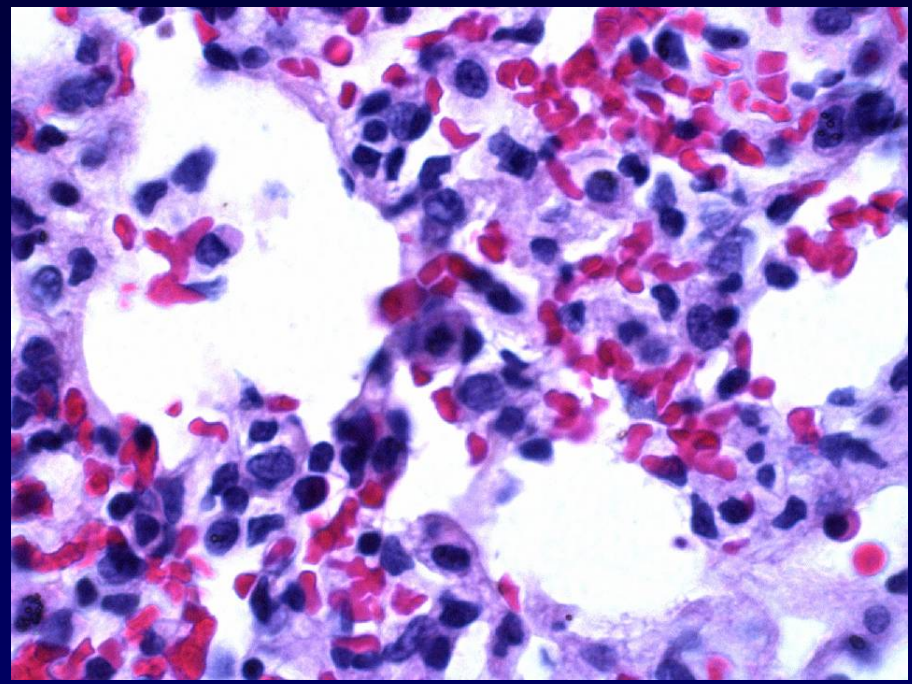
Control (200x)

Test (200x)

Inhalation study: Lung Tissue (H&E stain)

Alveolar Sac / Alveolar duct





Control (1000x)

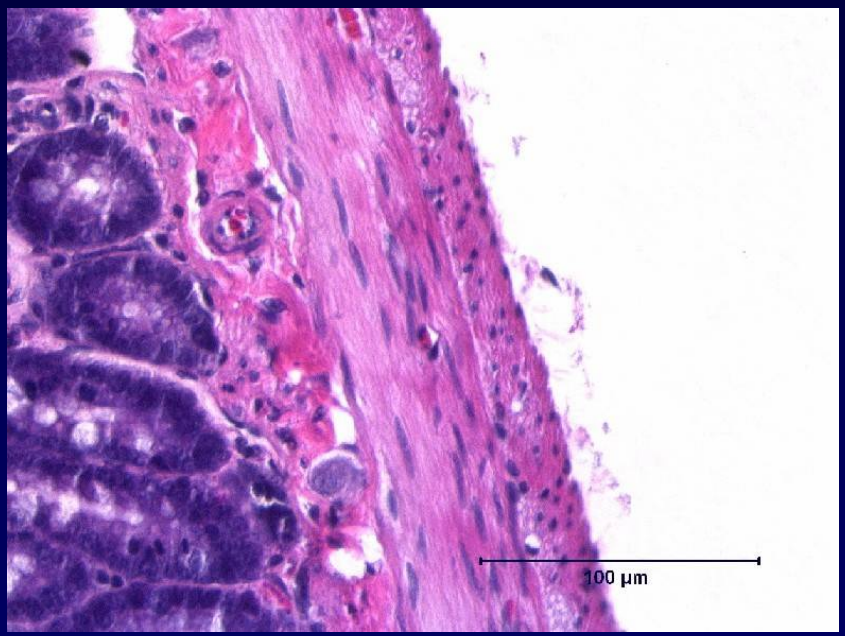
Test (1000x)

Dark spots are nuclei of endothelial and connective tissue cells. Red spots are red blood cells. No detectable difference.

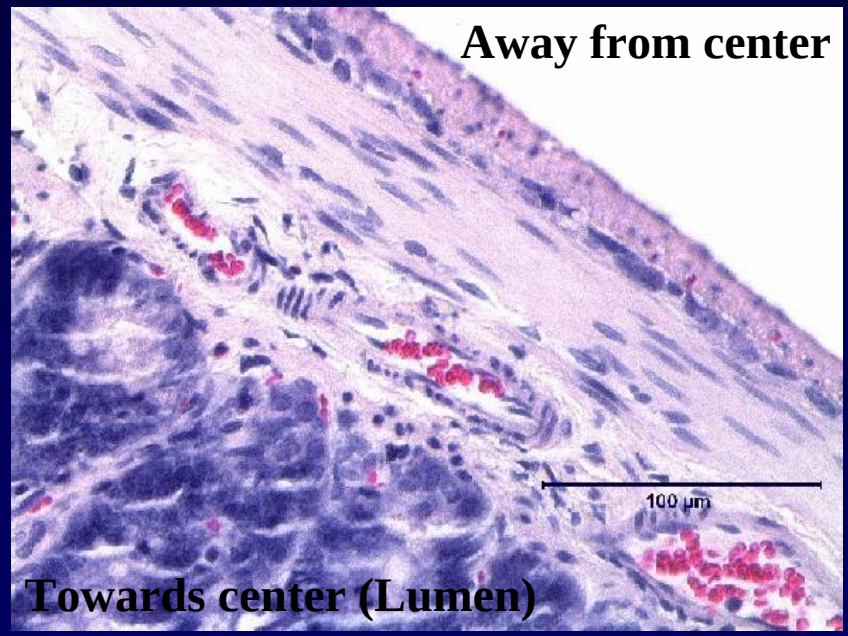
Ingestion Studies: FITC-labeled Mannan-NP

Oral Ingestion: Small Intestine Tissue (H&E stain) 72 hr.

Transverse sections



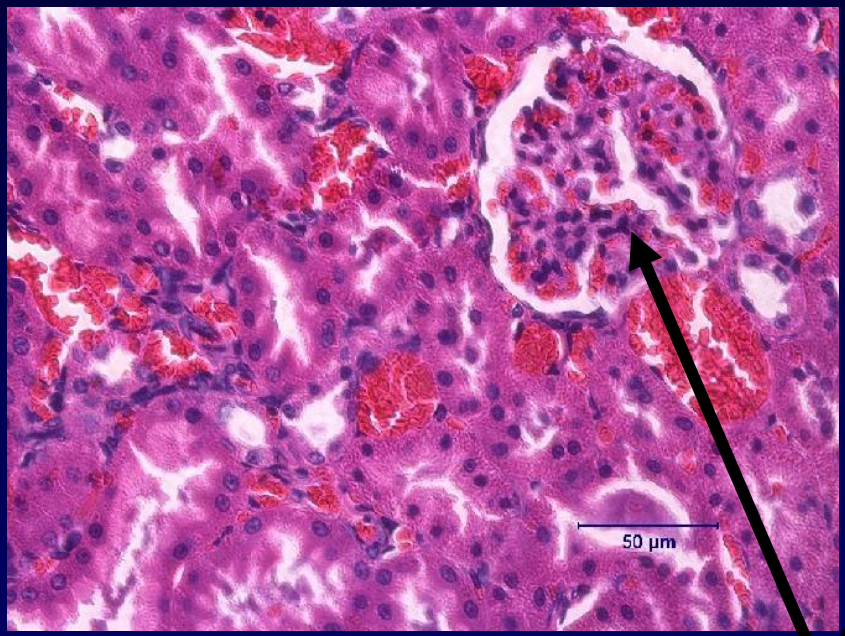
Control (400x)



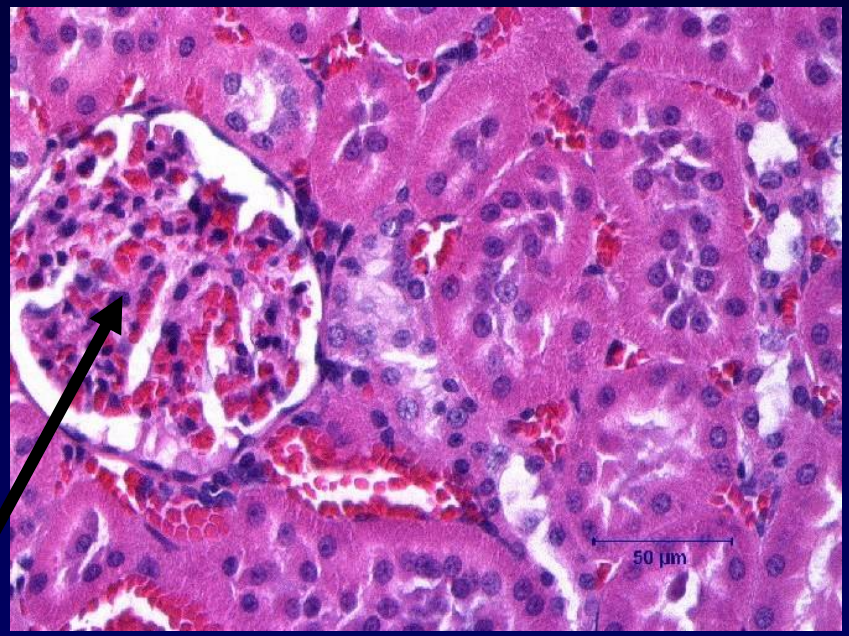
Test (400x)

No apparent difference.

Oral Ingestion: Kidney (H&E stain) 72 hr.



Control (400x)

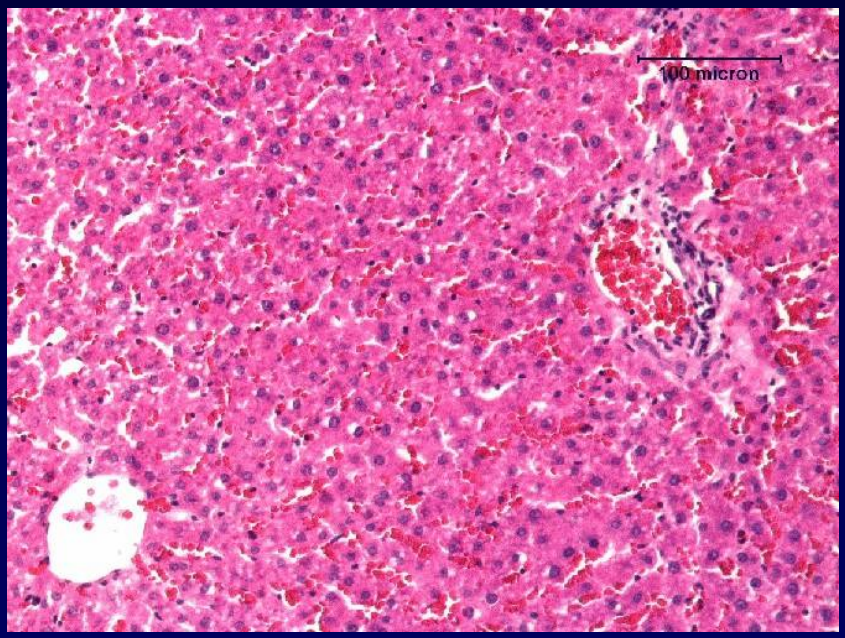


Test (400x)

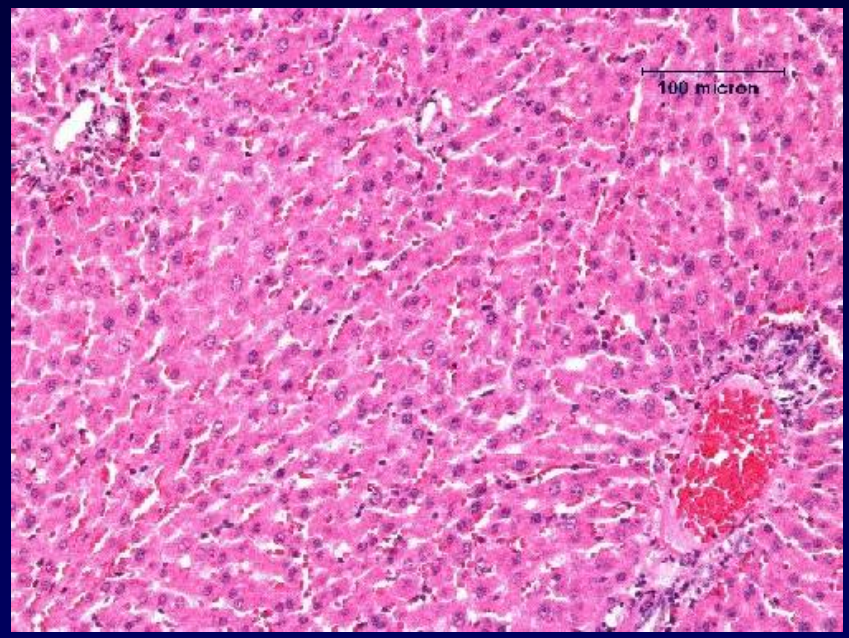
Glomerulus

No apparent difference.

Oral Ingestion: Liver (H&E stain) 72 hr.



Control (200x)



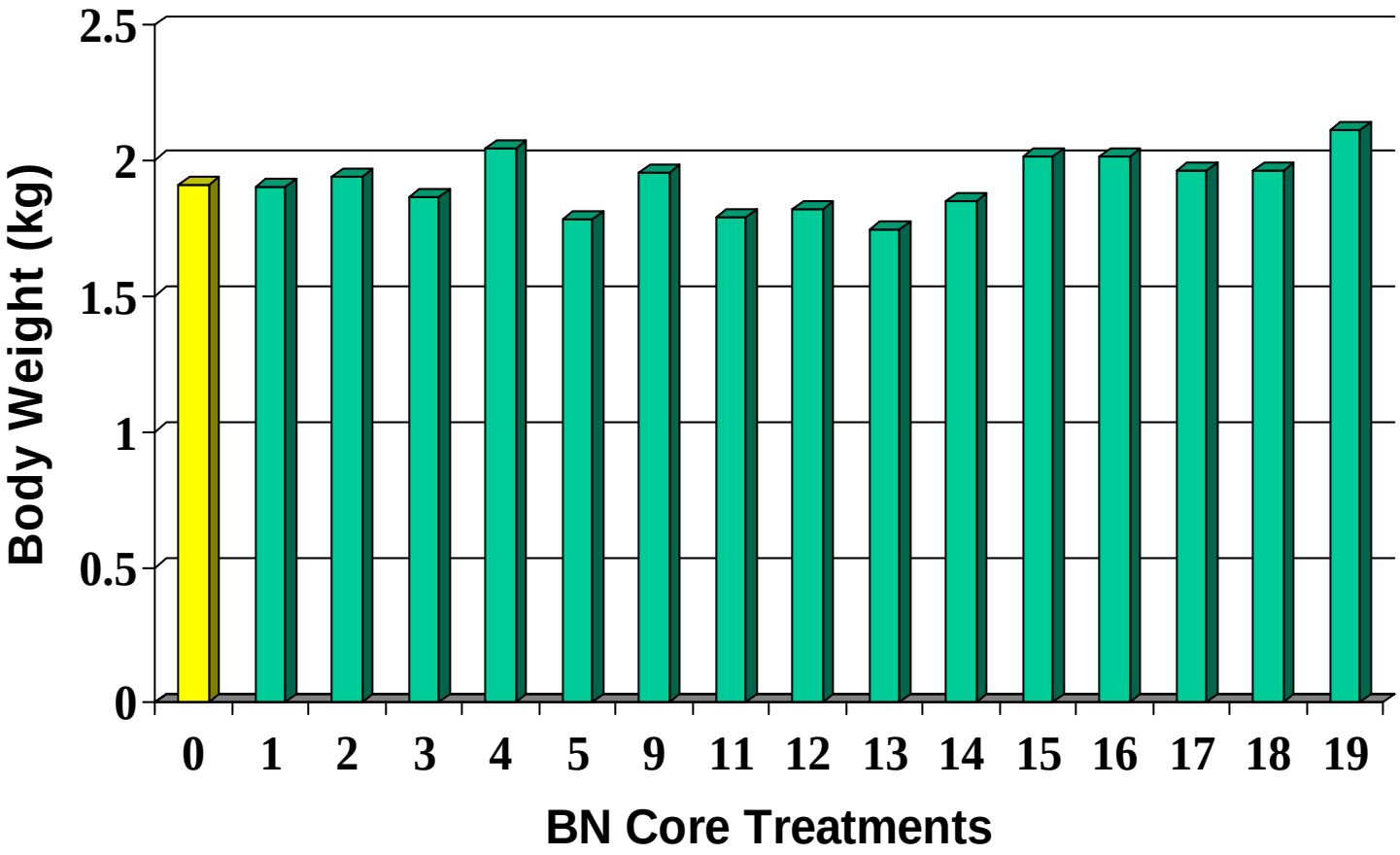
Test (200x)

No apparent difference.

Poultry Studies

- 1-2 poults/pen gavaged with 0.1, 0.5 or 1.0 mL per day of core-PEG nanoparticles, 2wt.%.
- 3 control poults/pen gavaged with distilled water
- Body weights at 1, 3 and 6 wk; observation to 14 wk
- Commercial feed and water *ad libitum*

Poult Performance: 6-week Body Weight



No significant effect of nanoparticles on poult body weight.

Concluding Remarks

- In vitro & in vivo studies conducted with polystyrene-based nanoparticles.
- No adverse cellular response for dermal fibroblast cells.
- No apparent adverse tissue response from dermal, ocular, inhalation, or ingestion routes of exposure.
- No adverse growth response from poultry studies.
- Further in vitro and in vivo studies planned.

Acknowledgements

- **USDA for funding support**
- **Collaborators:**
  - **Clemson University**
    - **F.J. Stutzenberger, T.-R.J. Tzeng, P.G. Luo, Dept. of Microbiology**
    - **Y.-P. Sun, L. Qu, S. Taylor, Dept. of Chemistry**
    - **S. Molugu, L. Jenkins, Dept. of Bioengineering**
    - **K. Bryant, J. Rodgers, Dept. of Envir. Toxicology**
  - **North Carolina State University, Dept. of Poultry Science**
    - **Jesse Grimes, B.W. Sheldon, J.L. Franklin, & M.J. Wineland**